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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: **SYSTEMS FOR GENE TARGETING AND PRODUCING STABLE GENOMIC TRANSGENE INSERTIONS**

(57) Abstract: The novel germ-line transformation systems disclosed in this patent application allow the physical deletion of transposon DNA following the transformation process, and the targeting of transgene integrations into predefined target sites. In this way, transposasemediated mobilization of genes-of-interest are excluded mechanistically and random genomic integrations eliminated. In contrast to conventional germ-line transformation technology, our systems provide enhanced stability to the transgene insertion. Furthermore, DNA sequences required for the transgene modification (e.g. transformation marker genes, transposase or recombinase target sites), are largely removed from the genome after the final transgene insertion, thereby eliminating the possibility for instability generated by these processes. The RMCE technology, which is disclosed in this patent application for invertebrate organisms (exemplified in *Drosophila melanogaster*) represents an extremely versatile tool with application potential far beyond the goal of transgene immobilization. RMCE makes possible the *targeted* integration of DNA cassettes into a specific genomic loci that are pre-defined by the integration of the RMCE acceptor plasmid. The loci can be characterized prior to a *targeting* experiment allowing optimal integration sites to be pre-selected for specific applications, and allowing selection of host strains with optimal fitness. In addition, multiple cassette exchange reactions can be performed in a repetitive way where an acceptor cassette can be repetitively exchanged by multiple donor cassettes. In this way several different transgenes can be placed precisely at the same genomic locus, allowing, for the first time, the ability to eliminate genomic positional effects and to comparatively study the biological effects of different transgenes.

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INTERNATIONAL SEARCH REPORT

International application No.

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A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C12N 05/06, 05/10; A01K 67/00, 67/033; C12N 21/00

US CL : 435/355; 800/13, 21

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/355; 800/13, 21

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 01/66717 A2 (THE UNIVERSITY OF UTAH) 13 September 2001 (13.09.2001), entire document.	1-48
A	US2002/0094575 A1 (SUZUKI) 18 July 2002 (18.07.2002), entire document.	1-48
A	US6,287,863 B1 (HODGSON) 11 September 2001 (11.09.2001), entire document.	1-48

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.*** Special categories of cited documents:****"A"** document defining the general state of the art which is not considered to be of particular relevance**"E"** earlier application or patent published on or after the international filing date**"L"** document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)**"O"** document referring to an oral disclosure, use, exhibition or other means**"P"** document published prior to the international filing date but later than the priority date claimed**"T"**

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